# Supplementary information:

**Surface modification of graphene nanopores for protein translocation**

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## 1. Graphene transfer

After CVD growth, a PMMA (950 A4 from Microchem) layer was spin-coated at 3000 rpm on the resulting multilayer graphene attached to the $Ni/SiO_2/Si$ wafer and baked at $115^oC$ for 2 minutes in an oven. The 300 nm thick Ni layer was then etched in 1 M $FeCl_3$ (in 3% HCl) solution overnight leaving the resulting graphene/PMMA sandwich floated on the solution surface while the $SiO_2/Si$ substrate sunk to the bottom of the Petri dish. By gradual replacement of the etching solution in the Petri dish with 3% HCl by a syringe, the residual metal particles and salt were dissolved and removed. After 60 minutes, HCl was gradually replaced with DI water and the receiving substrate was placed underneath the sandwich. Eventually, the water was drawn out by a syringe leaving the graphene/PMMA layer on the receiving substrate. The sample was then dried at $60-70^oC$ on a hotplate and kept in ethanol until use. Right before TEM, the sample was rinsed with dichloromethane and acetone to remove PMMA. The process was finished by heating on a hot plate at $80\ ^oC$ for 15 min.

## 2. The characterization of graphene after oxygen plasma treatment

We performed the contact angle measurements and Raman spectroscopy analysis of a similarly prepared graphene on $SiO_2$ (1" × 1") after oxygen plasma treatment. The oxygen plasma was generated by a plasma cleaner (Harrick plasma, Ithaca, NY) at 7.2 W RF power in a quartz vacuum chamber with a flow of pure oxygen at 550 mTorr and during 30 s. Figure S1a shows the contact angle measurements by a commercial instrument (EasyDrop, Kruss USA, NC). The contact angle is reduced from 91 $^o$ to 40 $^o$ after 30 s oxygen plasma treatment. For the Raman spectroscopy, we measured three different spots of different contrast on each graphene surface. As shown in Figure S1b, the G and 2D band peaks have different ratios corresponding to different number of layers but no D band peak is visible before the treatment. After 30 seconds oxygen plasma treatment, the D band peak near 1320 $cm^{-1}$ appeared, indicating the introduction of defects. Longer treatment showed no further change in either the contact angle or the D band intensity.

Figure S1c shows the pH dependence of the ionic current through an oxygen plasma treated graphene nanopore.
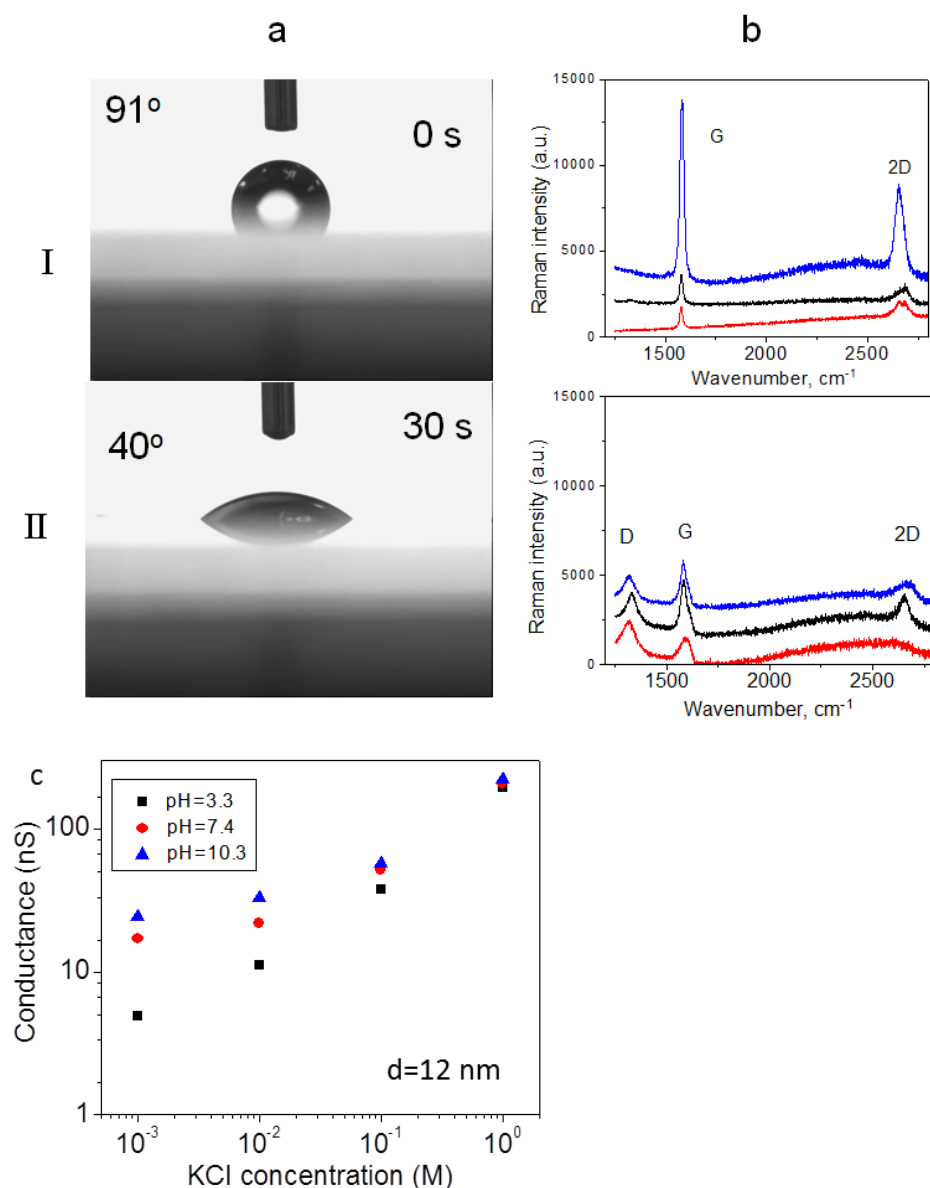
**Figure S1.** (a) Contact angles for graphene before (I) and after 30 s oxygen plasma treatment (II). (b) Raman spectra for graphene before (I) and after 30 s of oxygen plasma treatment (II) at three spots of different contrast. The baselines were shifted vertically for clarity. (c) The pH dependence of ionic current through a 12 nm diameter graphene nanopore after 30 s of oxygen plasma treatment.

### 3. EIS calibration of graphene chemical modification

For electrochemical impedance spectroscopy (EIS) analysis, the graphene was similarly transferred onto $SiO_2$/Si substrate (not suspended). The EIS measurements were carried out on an electrochemical workstation (CHI760D, CH Instruments, Inc, USA) using a three-electrode scheme in a home-built Teflon electrochemical cell at room temperature with Pt wire, Ag/AgCl

electrode and graphene on $SiO_2/Si$ substrate (the exposed area 3.14 $mm^2$) as the counter, reference, and working electrodes, respectively. Aqueous solution of 5 mM $K_3Fe(CN)_6$ and 5mM $K_4Fe(CN)_6$ in 0.1 M KCl buffered by PB (pH=7.0, typically 1mM) was used in the measurements. An alternating (AC) modulation (5 mV amplitude) was scanned in the $0.1$-$10^4$ Hz frequency range on top of a constant potential E = +0. 219 V, both applied to the graphene. This potential is close to the half redox potential of the redox probe ($Fe(CN)_6^{-3/-4}$) in such solution. The EIS data were fitted by the Complex Non Linear Least Square (CNLS) method based on a simple equivalent Randle circuit (inset of Figure S2a) using a Matlab (MathWorks Inc., USA) program to extract the equivalent circuit parameters, especially the charge transfer resistance $R_{ct}$. The exponent of the constant phase element (CPE) is close to 0.7 for all cases.

The values of $R_{ct}$ can be extracted from the fitting and crudely correspond to the diameter of the semicircle in the Nyquist plot. Greater $R_{ct}$ can be interpreted as better surface coverage by adsorption of molecules that hinder access of the redox probe to graphene. Figure S2 shows the Nyquist plots for C16 and DPPE-PEG750 modified graphene surfaces. The inset in Figure S2b shows the Nyquist plot for oxygen plasma treated graphene. After additional modification with C16, the $R_{ct}$ increased from 18 K$\Omega$ to 65 k$\Omega$ and a much more dramatic change from 3 k$\Omega$ to 388 k$\Omega$ after DPPE-PEG750 modification on a different sample. The modified graphene surfaces were then incubated with ferritin with a concentration of 0.47 mg/mL in 10 mM PB (pH 7.0) at room temperature for 90 minutes. After incubation, the surfaces were rinsed 3 times with DI water. As shown in Figure S2, the $R_{ct}$ increased from 65 k$\Omega$ to 95 k$\Omega$ for C16 modified graphene and from 388 k$\Omega$ to 407 k$\Omega$ for DPPE-PEG750 modified graphene. No significant changes in capacitance (CPE) were observed. We repeated the EIS measurements 3 times for each system and observed slight variation of $R_{ct}$ after oxygen plasma but consistently similar trend for its changes upon further modifications.
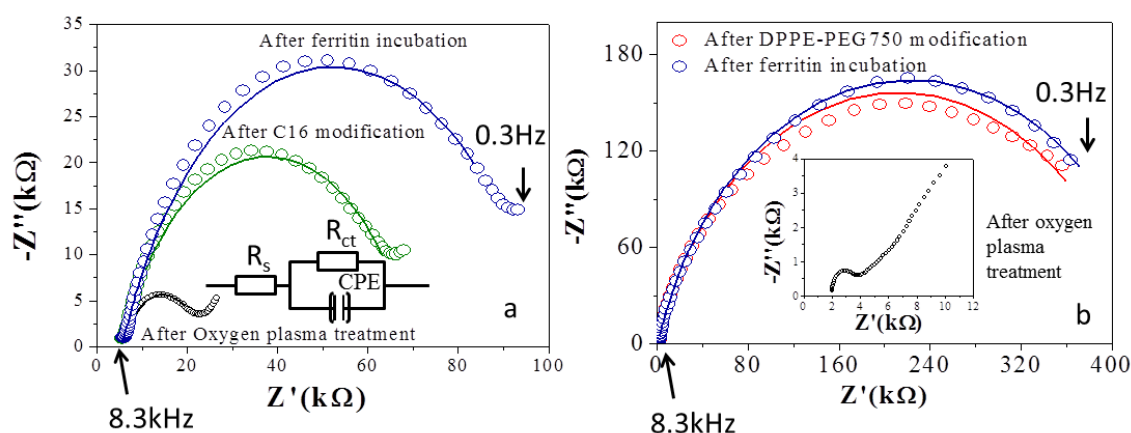


**Figure S2** Electrochemical Impedance Spectroscopy (EIS) results for different surface modifications. The Nyquist plots for C16 modified graphene (a) and DPPE-PEG750 modified graphene (b). The inset in (a) is the equivalent circuit, where $R_s$ is the solution resistance, $R_{ct}$ is the charge transfer resistance and CPE is the constant phase element. The Warburg impedance is

ignored here because its contribution is very small, which is different from the Nyquist plot for graphene after oxygen plasma treatment seen in the inset in (b). The open circles are experimental data and the solid lines are fitting results.

## 3. Gold nanoparticles translocation experiments

The translocation of 10 nm diameter gold nanoparticles (NPs) was observed in 5 nanopore devices after oxygen plasma treatment. Here we show the results from one nanopore device, with the pore diameter about 15nm (Figure S3e). We only observed current spikes at positive bias and only when the bias is higher than a threshold bias (0.55V in this case). However, the threshold bias is different for different nanopores. The width and amplitude of the spikes varied dramatically, couple of orders of magnitude for the latter.
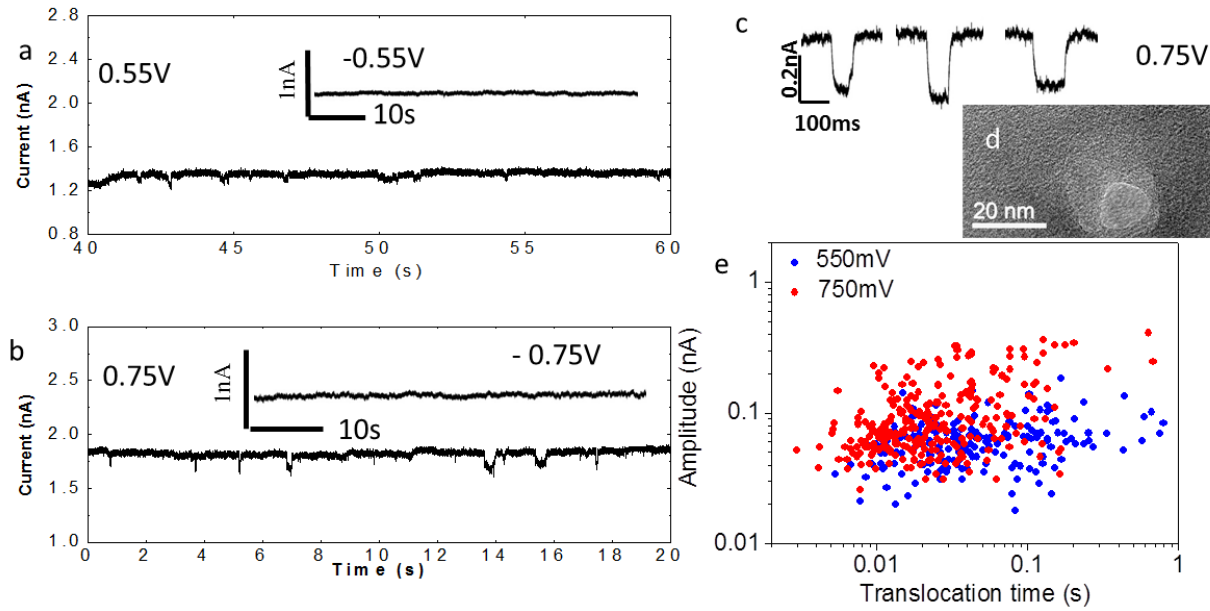


**Figure S3.** The current-time traces at 0.55V (a) and 0.75V (b) when 4.7nM 10nm Au NPs are added into the *cis* reservoir. The insets show the traces at negative biases. (c) Three representative spikes at 0.75V. (d) The TEM image of the nanopore. (e) A scatter plot (log-log) of the current spike amplitudes versus the spike widths (translocation times) at different biases.